

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Contribution to our knowledge of the physiology and metabolism of endurance horses

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/2494> since 2018-09-04T15:06:38Z

Publisher:

Elsevier BV:PO Box 211, 1000 AE Amsterdam Netherlands:011 31 20 4853757, 011 31 20 4853642, 011

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

Contribution to our knowledge of the physiology and metabolism of endurance horses

Domenico Bergero^{a,*}, Anna Assenza^b, Giovanni Caola^b

^a*Department of Animal Production, Epidemiology and Ecology, Faculty of Veterinary Medicine, University of Torino,
Via Leonardo Da Vinci 44, 10095 Grugliasco, Torino, Italy*

^b*Department of Morphology, Biochemistry, Physiology and Animal Production, Faculty of Veterinary Medicine, University of Messina.
Polo Universitario dell'Annunziata, 98158 Messina, Italy*

Abstract

The functional and metabolic processes of the endurance horse are discussed, based on personal experience and the scientific literature. Research on the energetic and physiological aspects of prolonged effort is reviewed, as regards the performance structure, described by Neumann, for human physiology.

Experimental data from our previous surveys on endurance horses, competing at different levels, are also presented and discussed.

The results on amino acid levels in blood, and related metabolic pathways, during endurance events, lead to speculation on the effects of the race distance on metabolic processes during long-lasting low-intensity exercise. In fact, the event distance has a significant influence on amino acid mobilization and their use as energy sources. In human athletes, the disappearance of some amino acids from blood circulation is linked to the onset of central fatigue. The effect could be similar in endurance horses; therefore, the correct intake of amino acids, and a proper feeding strategy, could improve performance.

Data are also provided on dehydration and oxidative stress associated with endurance work.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Horse; Endurance; Exercise physiology; Nutrition

1. What “endurance” means in competition

Endurance horses compete in races that can be classified as low-intensity long-lasting trials. The first

modern endurance ride was held in 1955 in California, from Lake Tahoe to Auburn (100 miles). Today, most endurance rides range from 30 to 160 km, or 100+100 km to be run over 2 days, or 500 km to be run over 5 days (Duren, 2000; Sosa Leon, 1998).

Most breeds have been tested and used for endurance races; the most competitive are Arabian or Arabian crosses due to their muscle fibre compo-

* Corresponding author. Tel.: +39 11 670 9207; fax: +39 11 670 9240.

E-mail address: domenico.bergero@unito.it (D. Bergero).

sition, but other breeds, including Thoroughbred, Quarter Horses, Mustangs, Appaloosas, Morgans, Standardbred, and even Mules, have been used successfully (Duren, 2000). Arabian horses, compared to Thoroughbreds, are better adapted to endurance work because of their superior oxidative capacity (Prince et al., 2001). Arabians and Anglo-Arabians seem to have lower lactate concentrations, compared to Andalusians, at speeds up to 25 km/h (Castejon et al., 1994), thus, showing a better adaptation to long-distance, low-intensity work.

Endurance competitions are extremely difficult from a metabolic point of view, and for this reason, they are subjected to very strict veterinary controls to spare the horse's health. In an overview of 7117 starts in international (Eldric) races, only 50% of the subjects completed the ride, and 30% were eliminated: 63% because of lameness, 24% for metabolic reasons, and 13% for other causes (Burger and Dollinger, 1998). Metabolic problems, therefore, cause the elimination of 7.2% of horses starting international races, but some are retired for the same reasons between two veterinary gates during the race, while others have problems after the final veterinary examination.

For this reason, the correct metabolic management of the endurance horse is of the utmost importance, together with the correct prevention and treatment of osteo-articular pathologies.

2. Physiology overview of endurance effort

In sport, apart from the species involved, management is normally understood to mean the combination of actions aimed at obtaining maximum athletic performance, including economic, medical, as well as ordinary athletic factors (athletic evaluation, training regime, correct feeding).

From a physiology effort point of view, correct management is based on knowledge of the metabolic and functional processes involved in the particular athletic discipline.

In other words, it is of the utmost importance to understand which metabolic pathways are involved, and which physiological adaptations are induced by different type of exercise. In fact, different competitions can be classified according to criteria based on

fuel utilization, work intensity, and work duration. This, in turn, allows the planning of an appropriate feeding regime. Indeed, these two aspects are closely related; in particular, for endurance work, which is very heavy from a metabolic point of view, as described here.

In sport exercise physiology, the word “endurance” defines the physical and mental capacity to withstand fatigue (Weineck, 1990).

The general concept of endurance can also be divided into different categories, as follows:

- General or local endurance, according to the proportion of muscle mass involved in the physical exercise (approximately 1/7–1/6 of the total voluntary muscle mass);
- Basic or specific endurance, according to either the general endurance capacity to perform exercises or the ability to compete in a particular sport discipline;
- Aerobic or anaerobic endurance, according to the fuel utilized and the energy metabolism involved;
- Static or dynamic endurance, according to the type of muscle contraction;
- Endurance to strength, explosive strength and velocity, according to the kind of muscle contraction activated.

It is also usual to divide the different sport competitions according to the duration of the effort: in this way, we can classify the endurance required as: short (from 35 s to 2 min), medium (over 2 min to under 10 min), and long-lasting (from 10 min to 6 h and more) (Neumann, 1990).

This classification is extremely useful, because it overcomes the problems raised by analytical classifications, and, more importantly, it permits the integration of the different adaptation processes, which took place in the whole organism, according to the duration of the required activation.

In endurance trials, therefore, it is extremely important that the principle metabolic processes reach a steady-state. We repeatedly measured the metabolic activity of horses competing in endurance trials, by recording the heart rate during the whole race. A sample of the measurements is given in Figs. 1 and 2, of a horse competing in a 72-km ride, completed successfully. The mean heart rate was, on this

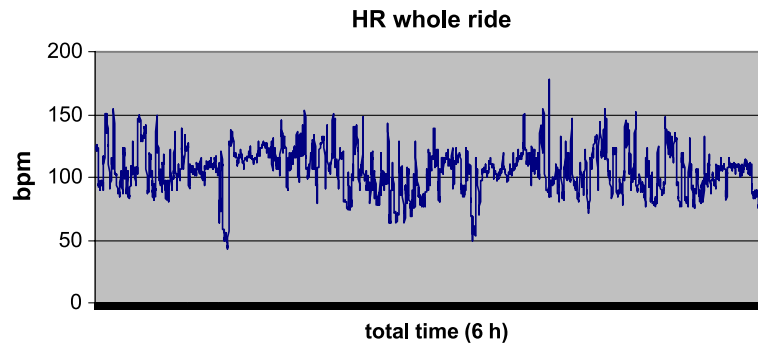


Fig. 1. Recorded heart rate of a horse competing in a 72-km endurance ride.

occasion, about 62% of the measured anaerobic threshold (Pinna et al., 2004), which is in accordance with the definition of long-lasting endurance.

Another important aspect of sport physiology is a knowledge of the different external (biomechanics and kinematics) and internal (metabolism and general functions) factors involved in the physical effort, and which can interfere with final performance results. These factors can be coupled together as the “performance structure” (Neumann, 1990), which is, from a physiological point of view, related to the adaptation processes, which in turn, are the final goal of physical training. To elucidate the performance

structure, the final target of the specific training must be well defined. Therefore, this methodological approach, well known and documented in human athletes, has been adapted and applied to the athletic horse, because it is one of the ways to achieve effective management of the athletic horse, in general, and of the endurance horse, in particular (Assenza et al., 1997).

3. Energy supply

During prolonged effort, the main sources of energy production (ADP phosphorylation) are non-structural carbohydrates and fats. Amino acids, however, play an increasingly recognised role, particularly, branched chain amino acids (BCAA), which are important from both a quantitative (3–15% of the total energy supply is supplied by amino acid catabolism; Holmann and Mader, 1999), and a qualitative point of view. In fact, branched chain amino acids are implicated in the prevention of the onset of central fatigue (Newsholm et al., 1992; Assenza et al., 2000, 2002; Caola, 2001).

The relative percentage utilization of the two main energy sources (carbohydrates and fat) varies according to the intensity and duration of the exercise.

Muscle glycogen is another important energy source; a dramatic decrease in its muscle content has been reported in horses competing in endurance rides (Snow et al., 1981).

The reduction of muscle glycogen is linked, together with an increase in lactic acid (LA) and a decrease in glucose blood levels (glycaemia), to the onset of peripheral fatigue (Newsholm et al., 1992).

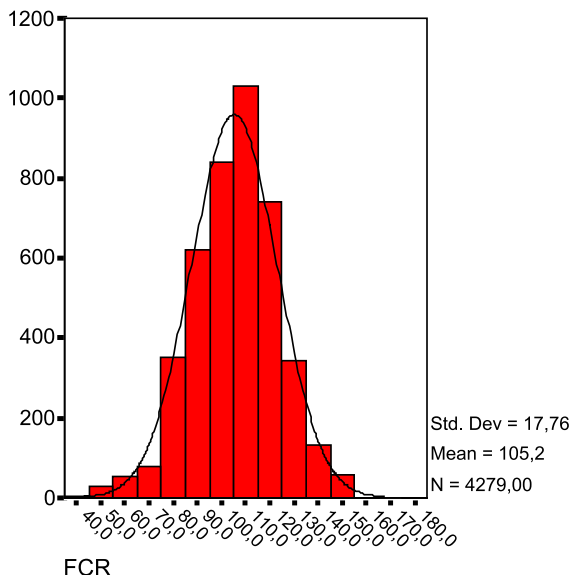


Fig. 2. Heart rate frequency histogram of a horse competing in a 72-km endurance ride (Mean, standard deviation, and number of observations are indicated).

It is clear that fatty acid (either released from muscle triglycerides or circulating in the blood, as non-esterified fatty acids, NEFA) metabolism plays a significant role in energy supply, thereby increasing exercise duration (Snow et al., 1983).

The final effect of increased fatty acid utilization is, in fact, a saving in the utilization of muscle glycogen. Other energy sources, e.g. those connected with the alanine and glutamine cycles, or the oxidation of ketone bodies, probably play a lesser, but by no means a negligible, role (McGilvery, 1975).

4. Branched chain and other amino acids

As previously mentioned, fatigue can arise from both peripheral and central causes. For the endurance horse, as for marathon runners, amino acid catabolism could have an important role in the onset of central fatigue, and, together with hypoglycaemia, could involve the onset of both kinds of fatigue.

During exercise, the utilization of BCAA as a fuel source in muscle fibres involves a decrease in their blood plasma concentration, leading to a decrease in the blood BCAA/free tryptophan ratio. At the same time, the rise of NEFA levels in the blood involves the increased utilization of albumins as carrier proteins for the same NEFA, and decreased utilization as a tryptophan (Try) carrier. Therefore, the plasma level of free tryptophan increases, and can pass through the

blood–brain barrier. This is facilitated by a decrease of BCAA in plasma, making a large number of carriers available to cross the barrier.

In the brain, an increase in Try availability involves an increase in serotonin (a neuro-transmitter, involved in the rise of fatigue) production (Dillon, 1992).

Recent studies on endurance horses seem to indicate that BCAA catabolism is similar to that found in human athletes; in particular, the aspects related to duration of workload (Assenza et al., 2000, 2002; Arcigli et al., 2002). Data on the changes in tryptophan and BCAA levels in blood, during endurance rides, are shown in Tables 1 and 2.

Other metabolites are also implicated in the onset of fatigue, for example, inosine monophosphate, a product of adenine nucleotide degradation (Essen-Gustavsson et al., 1999).

In previous studies (Bergero et al., 2003; Assenza et al., 2004), we stated that during a 32-km ride, that included a steep slope (819-m difference in height), the levels of some free amino acids in blood serum increased significantly. Tyrosine increased after the slope compared with the finish, and after the finish compared with just before the start. Phenylalanine and tryptophan increased both after the slope and the finish compared with just before the start.

A second group, competing in a 72-km endurance ride, was also sampled just before and just after the race.

Table 1

Free amino acids in blood serum: basal values measured immediately before the start of various endurance rides

Amino acid	Distance of endurance event (km)				
	20	32	52	62	72
Arginine	54.7±18.7	62.5±5.8	90.2±31.0	64.3±17.0	102.6±3.4
Asparagine	34.3±12.2	34.7±1.4	43.6±11.3	44.7±18.5	55.2±11.8
Methionine	17.7±3.1	39.7±5.6	30.8±6.9	24.0±2.6	36.4±4.9
Alanine	331.3±154.5	159.6±3.1	443.6±184.6	520.0±237.1	382.0±29.3
Glycine	94.5±40.8	31.8±3.2	51.2±77.1	52.0±8.7	76.6±12.0
Isoleucine	70.0±33.9	87.1±11.0	112.8±59.1	100.7±2.3	132.4±14.5
Lysine	95.5±10.1	117.3±3.7	211.8±64.2	134.7±16.7	133.0±0.9
Leucine	98.8±8.0	144.1±21.9	129.6±34.8	115.0±38.2	136.4±90.6
Valine	142.5±27.5	150.6±20.2	171.4±37.8	148.7±21.0	175.6±17.4
Tyrosine	16.9±1.6	90.3±3.8	17.0±3.2	24.8±3.9	31.5±0.6
Phenylalanine	11.2±1.9	57.5±6.2	13.2±5.2	16.9±2.6	23.2±1.6
Tryptophan	19.7±3.6	21.3±1.8	19.2±5.22	22.4±6.6	15.4±2.7
Hystidine	104.7±17.3	78.2±2.8	124.0±34.5	80.7±5.1	94.6±4.6
Ornitine	35.0±6.9	58.2±4.0	60.6±42.1	82.3±25.1	92.6±3.5

Table 2

Free amino acids in blood serum: post-exercise values measured immediately at the end of various endurance rides

Amino acids	Distance of endurance event (km)				
	20	32	52	62	72
Arginine	122.3±28.8	128.0±7.7	93.2±52.2	139.0±44.0	68.4±6.7
Asparagine	55.0±16.5	78.3±4.3	59.2±32.2	44.7±18.5	31.8±1.5
Methionine	29.0±5.8	75.7±11.4	55.8±34.7	69.6±38.3	23.0±2.4
Alanine	308.7±170.7	339.1±20.9	449.2±260.4	490.3±149.8	236.8±47.9
Glycine	105.5±30.9	78.7±11.0	101.0±54.0	61.3±6.2	39.6±7.0
Isoleucine	92.3±52.7	108.2±24.7	115.8±50.0	130.3±48.4	102.4±32.2
Lysine	155.7±83.5	204.1±12.4	175.2±42.1	222.3±67.5	132.2±8.7
Leucine	152.8±10.9	294.0±71.7	278.8±92.5	210.3±75.9	69.8±21.7
Valine	240.6±65.3	281.8±75.5	254.8±57.0	175.0±39.0	123.6±17.7
Tyrosine	22.6±4.0	120.7±4.7	22.9±4.1	23.7±1.9	23.0±0.9
Phenylalanine	13.3±2.6	89.6±8.0	13.9±2.0	18.3±1.6	16.3±1.6
Tryptophan	23.3±4.9	39.6±3.9	12.9±4.9	15.9±1.1	30.8±8.4
Hystidine	104.8±25.2	148.5±14.3	124.6±49.0	110.0±24.3	65.2±3.7
Ornithine	79.7±16.8	172.2±30.1	282.4±150.9	196.7±22.5	65.8±5.9

The pre-ride levels of free amino acids were different between the two races, except for methionine and leucine. Differences between start and finish were found, in both race groups, for all amino acids examined (alanine, arginine, asparagine, glycine, isoleucine, histidine, leucine, lysine, methionine, ornithine, phenylalanine, tryptophan, tyrosine and valine), with the exception of asparagine, isoleucine, leucine and lysine for the 72-km ride. In blood serum, increased levels were recorded for the shorter race, and decreased levels for the longer ride. There was a significant increase in alanine, arginine, asparagines, phenylalanine and lysine levels only between samples taken at the start and the second sample taken at the top of the slope.

We also studied the variations of BCAA and Try blood serum levels in 30 endurance horses competing in rides varying in distance from 20 to 72 km. Blood samples were drawn from all horses just before and immediately just after the end of the ride. Samples were analysed for leucine (Leu), valine (Val), isoleucine (Iso) and Try levels. Significant differences were recorded, for the various distances, for Leu, Val, Iso, Try, Try/BCAA ratio; the same trend was found for Val and Leu, between samples taken at the start and at the end of the race. The main observation was an increase in BCAA levels for all distances, except the 72-km ride; for Try, a significant increase was present in all races, except the 50-km distance. The Try/BCAA ratio decreased in the 20- and 50-km

races, and increased in the others. The data confirm that long-distance exercise involves a mobilization of BCAA. The utilization of BCAA seems to be important in prolonged exercise: in the 72-km ride, we observed a decrease in BCAA blood serum levels, while a major role of Try was indicated by increased levels, resulting in a rise of the Try/BCAA ratio.

An overview of the studied parameters is given in [Tables 1 and 2](#). As a general observation, it should be noted, that amino acid levels in blood serum increased during races up to 52 km long, and decreases for the 72-km rides. Isoleucine, on the contrary, remained steady, while the level of alanine in blood started to decrease at 32 km, and continued to decline at 72 km.

Race length has, therefore, a significant impact on blood serum amino acid mobilization and uptake; in the shorter race, the increase expresses mobilization only, whereas in the longer race, the decrease can be considered as the effect of the onset of amino acid catabolism.

5. Fat and antioxidant status

Endurance horses, performing aerobic work, use mostly NEFA as a source of ATP regeneration. Despite the fact that NEFA come from hindgut fermentation and the mobilization of stored fat, the use of fat-enriched diets can be beneficial for long-distance exercise in horses. Moreover, fat is a good

source of important fat soluble nutrients, e.g. essential fatty acids and fat soluble vitamins; in particular, vitamin E, which has antioxidant properties. In fact, horses fed fat-enriched diets can mobilize and utilize fat more efficiently during long-distance exercise (Pagan et al., 1987). Fat-adapted horses also have a higher rate at which lactate began to increase sharply during incremental exercise, and lower LA blood concentrations during aerobic work (Kronfeld et al., 2001). The adaptation of horses to fat-enriched diets does not alter the rate of muscle glycogen recovery (Hyypä et al., 1999). Fat predilection can, however, affect daily heat load, feed intake, faecal output, and water requirements, under hot and humid conditions (Kronfeld, 1996), and involves a higher percentage of absorbed water, even though it implies a higher sweat loss (Mathiason-Kochan et al., 2001).

Many fat sources are added to the diet of endurance horses; corn oil and soybean oil are the most commonly used (Duren, 2000). Spangfors (2000) advises the use of a fluid vegetable fat with 30% linoleic acid at a 5–6% rate in the ration. For endurance horses, Duren (2000) proposes a grain diet, top-dressed with 7–10% fat, as optimal, and an added fat limit level of 20%, as the threshold for palatability.

Polyunsaturated fatty acids, which form a large proportion of fats added to the diet of endurance horses, have a structural role in cell membranes, and are selectively incorporated into the different tissues. They are more often used for constructional rather than for energetic purposes. Plasma NEFA composition, which is related to dietary supply, does not reflect the composition of the different tissues. For example, while feeding a diet rich in linolenic acid, the fatty acid composition of skin differed from that of blood serum (Bergero et al., 2002a), thus showing selective incorporation.

Reactive oxygen species (ROS) play an important role in skeletal muscle damage and inflammation after strenuous exercise. It is recognised, for example, that physical exercise can modify malonylaldehyde and glutathione content of horse's blood (Chiaradia et al., 1998). During endurance rides, the high demand for antioxidants to scavenge ROS decreased the levels of vitamin C and glutathione in blood. On the other hand, the blood level of vitamin E increased, due to its release from adipose tissue, associated with fat mobi-

lization during the race (Hargreaves et al., 2001). For this reason, the role of dietary antioxidants, for endurance horses, is well recognised and increasingly studied (Hargreaves et al., 2001). The dietary supplementation of horse diets with fats, containing large amounts of polyunsaturated fatty acids, in particular, can influence the oxidative status of horses. This can be modulated by adding vitamin E (Bergero et al., 2002b), even though studies on dietary utilization of antioxidants to reduce exercise-induced muscle damage are still under discussion (Sacheck and Blumberg, 2001). According to White et al. (2001), the administration of vitamin C to Thoroughbred horses could decrease oxidative stress produced by racing, but could not prevent muscle damage. Heat can increase oxygen radical production during exercise, but there is insufficient evidence to propose nutritional needs in these conditions (Burke, 2001).

The effect of the addition of a daily 20-g dose of an unsaturated fatty acid-rich oil from Purple Viper's Bugloss seeds on saddlehorses, fed a near-maintenance level diet of first-cut meadow hay/barley, has been studied by our group (Bergero et al., 2002a). The percentages of C16:0, C18:0, C18:1, C18:2 ω 6, C18:3 ω 3, C18:4 ω 3, C20:3 ω 6, C20:4 ω 3, C20:4 ω 6, C22:5 ω 3, C22:6 ω 3, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, ω 3 fatty acids, and ω 6 fatty acids, were determined in blood serum and skin. The ω 3/ ω 6 ratio was also recorded, and no differences were found due to the treatment. In the skin, a higher percentage of: C16:0, C18:1, C18:4 ω 3, monounsaturated fatty acids, ω 3 fatty acids, and saturated fatty acids was found, compared with blood serum; the ω 3/ ω 6 ratio was also higher in the skin. Lower percentages of C18:0, C18:2 ω 6, polyunsaturated fatty acids, and ω 6 fatty acids were also recorded in the skin, compared with blood serum.

Blood levels of fatty acids are not constant, according to values recorded in the literature, but some differences can be explained by the variation in diets used. In this study, the lack of significance in the effect of the oil can be explained by the low level in the addition of oil.

In another study (Bergero et al., 2004), six horses were used in a Latin-square design, in which three homogeneous groups were assigned three different dietary treatments for 1 month each. Control group: basic diet; Oil group: Basic diet+200 g/day oil rich in

γ -linolenic acid; Vitamin E group: basic diet+200 g/day oil rich in γ -linolenic acid+5 g/day α -tocopheryl acetate. At the end of each experimental period, blood samples were taken by jugular vein puncture. Serum oxidative status was evaluated by TBARs and d-ROMs assessment. Oxidative markers showed the highest mean values for the oil group, even if no statistically significant differences were found (Bergero et al., 2004).

6. Macro-minerals, water

Water and electrolytes supply is a key factor for endurance horses. The large intestine is the reservoir of the supply of these nutrients. It is important to focus on the role that high fibre diets have on sodium metabolism: retained sodium is significantly higher when horses are fed high-fibre diets (Spangfors, 2000).

Fluid and electrolyte loss, together with acid–base imbalance, and intramuscular glycogen depletion are the main nutritional causes of exhaustion that occurs in most equestrian sports, but it is more frequent in events that require endurance (Foreman, 1998).

Training has an important effect on the fluid, electrolytes and acid–base status of horses, both for intense and prolonged exercises. In particular, this effect has been studied in Thoroughbred trained for sprinting (Hyypä and Poso, 1998).

Requirements for water and its relation to work are also very important. Exercise conducted in a hot, humid environment may increase water requirements by 300%, giving a total daily water intake of 90 l (Duren, 2000). Moreover, endurance exercise influences body weight, body water, and packed cell volume in the horse, as shown in Table 3 (Bergero et al., 2001).

The endurance horse has demonstrated an enormous ability to compensate for fluid and electrolyte losses by using equilibrium mechanisms between extracellular and intracellular compartments. Fatigue and exhaustion may occur when compensatory mechanisms fail owing to severe water and electrolyte losses, and insufficient replacement (Flaminio and Rush, 1998).

Water loss, connected to endurance work, is a major concern. There is evidence that, even after over

night recovery and despite an apparent rapid return of plasma volume and ionic composition to near normal values, substantial depletion of body fluids and electrolytes persists after the completion of 50 or 100 miles endurance competitions (Schott et al., 1997).

Electrolytes are minerals that dissociate in solution into electrically charged ions (Na^+ , K^+ , Cl^- , Ca^{2+} , Mg^{2+}). They are lost with faeces, urine and sweat. Sweat loss of electrolytes, in particular, causes the onset of peripheral fatigue and weakness. For this reason, good nutritional management of endurance horses requires the replenishment of these losses by orally administered salts. Sweat loss of potassium and chloride is particularly important, where concentrations are greater than in plasma; substantial decreases of those minerals have been recorded during long-distance exercise (Rose et al., 1980). In periods of extreme sweating, the administration of 5 l of water with 30 g of NaCl, or twice as much of mixed electrolytes and 15 g sucrose or glucose, is recommended for a 500-kg horse (Frape, 1988).

At present, there are no data to support the concept of “electrolyte loading” of equine athletes by feeding supplemental electrolytes for several days prior to competition. The additional electrolytes are quickly excreted by the kidney within a few hours. Nevertheless, electrolytes administered in the few hours immediately before a prolonged exercise competition may be of benefit if adequate water is also ingested (Schott and Hinchcliff, 1998).

Water intake during endurance exercise seems to be improved by the use of hypertonic oral pastes (Dusterdieck et al., 1999), even if Nyman et al. (1996) report an adverse effect of this salt regime, thus advising the oral utilization of 0.9% NaCl saline solution.

Glycerol administration, as proposed for human athletes competing in different disciplines, seems to be ineffectual for the prevention of weight loss after recovery from a simulated ride, and increases urine electrolyte losses when administered with electrolytes (Dusterdieck et al., 1999; Schott et al., 1999).

Magnesium losses are prone to cause muscle problems in endurance horses. Because calcium and magnesium are chemically alike, both ions use the same uptake and transport mechanism in the body. In

Table 3

Measured and calculated parameters (means±standard deviation) in 12 endurance horses competing in a 32-km endurance ride

Weight	InW (kg)	FiW (kg)	WL (kg)	WL%	
	448±45	427±42*	20±5	4.6±1.0	
Packed cell volume	iPCV	fPCVf	ΔPCV		
	44±2	51±2*	6.5±3.1		
Fluids	TBW0 (l)	TBW1 (l)	Wl (l)	Wlex (l)	Wl%
	296±29	277±27*	18±5	15±4	6.2±1.2
Electrolytes	Na ⁺ (mmol)	K ⁺ (mmol)	Cl ⁻ (mmol)		
	2041±622	1029±266	3859±1000		

InW: initial weight; FiW: final weight; WL: weight loss; WL%: percent weight loss; iPCV: initial packed cell volume; fPCVf: final packed cell volume; ΔPCV: difference between final and initial packed cell volume; TBW0: initial total body water; TBW1: final total body water; Wl: water loss; Wlex: extracellular water loss; Wl%: percent water loss; Na⁺, K⁺ and Cl⁻: theoretical estimation of sodium, potassium and chloride sweat loss.

* Significant differences compared to initial values ($P<0.001$).

the competition between the two ions, calcium is commonly the winner, because its absorption is regulated by hormones and vitamin D. For this reason, magnesium deprivation can occur in diets containing normal amounts of this ion (Spangfors, 2000). Therefore, diets containing an excess of calcium (e.g. based on good quality alfalfa hay as the only forage source) must be avoided.

Replacement of fluids and electrolytes should be based on sweat losses occurring per hour of exercise, which, in most cases, will be 2–5 l, but can be higher (10–15 l) if exercise is performed in hot and humid conditions.

For rides of approximately 100 km or more, which are run at a slow pace (about 2–4 m/s), 2–5 l of fluid can be given per hours of exercise.

Research on multiday rides is limited to one study, in which daily body weight losses were recorder in an endurance competition of around 500 km over 5 days. In this kind of competition, fluid losses must be compensated during the ride, in the same manner as described for rides over 100 km, and it is important to supplement horses at the end of the ride, with the aim of returning fluid and electrolyte balance to normal (Sosa Leon, 1998).

From our previous experiences on this topic (Bergero et al., 2001), we studied a group of 12 endurance horses, Arabian stock, aged from 5 to 12 years, and competing in a endurance ride of 32 km, run at 8–14 km/h. All horses were weighted before and after the ride, and the heart index (HI) was measured at 152, according to the method of Clayton (1991) ($HI = \text{Temperature } [^{\circ}\text{C} \times 1.8] + 32 + \text{Rh}\%$).

In all horses, before and after the ride, packed cell volume (PCV) was measured, and the following parameters recorded:

- Total water at the start (TW0)
- Total water final (TW1)
- Water loss during the ride (Wl)
- Extracellular water loss during the ride (Wlex)
- Dehydration percentage

For these calculations, we used the recorded weights and the equations proposed by Andrews et al. (1994) and the assumptions of Demonceau (1992).

We were able, then, to calculate the theoretical losses of Na, K and Cl⁻, according to Demonceau (1992).

During the race, the packed cell volume changed from 44±2% to 51±2%. All results are summarized in Table 3. As a general comment, the large loss of electrolytes induced by the endurance work must be taken into account when adopting proper re-hydration strategies. In our study, for example, about 140 g NaCl was necessary, as a mean value, to recover from the calculated Na losses.

7. Conclusions

Endurance horses have special needs, and their management requires a deep knowledge of both fundamental and applied nutrition and physiology. To compete with success in these types of competi-

tion, correct training and nutritional regimes are necessary, and metabolic controls must be performed during training. If this protocol is applied, well adapted and trained horses can cope with this type of exercise. Even if endurance races are very tough, they represent a unique opportunity for technicians to assess the suitability of management strategies, and should be considered more as a training exercises in applied physiology rather than intimidating events.

References

- Andrews, F.M., Ralston, S.L., Sommardahl, C.S., Maykuth, P.L., Green, E.M., White, S.L., Williamson, L.H., Holmes, C.A., Geiser, D.R., 1994. Weight, water and cation losses in horses competing in a 3-day event. *J. Am. Vet. Med. Assoc.* 205 (5), 721–724.
- Arcigli, A., Assenza, A., Nicosia, S., Piccione, G., 2002. Modificazioni dei livelli ematici degli aminoacidi a catena ramificata in cavalli impegnati in gare ufficiali di fondo. *Atti SISVet* (in press).
- Assenza, A., Scrivano, P., Cullò, V., Caola, G., 1997. Verso una definizione della struttura della prestazione nel cavallo fondista. *Atti SISVet* (Soc. Ital. Sci. Vet.) 51, 531–532.
- Assenza, A., Pellegrini, L., Celona, B., Bergero, D., Caola, G., 2000. Aminoacidi a catena ramificata nel siero di sangue di cavalli durante gare ufficiali di fondo: un approccio preliminare. *Atti 2° Convegno "Nuove Acquisizioni in Materia di Alimentazione, Allevamento e Allenamento del Cavallo Sportivo"*, Campobasso, 13 Ottobre 2000, pp. 41–43.
- Assenza, A., Bergero, D., Tarantola, M., Piccione, G., Caola, G., 2002. Blood serum branched chain amino acids and tryptophan modifications in horses competing in long distance rides of different length. *Book of abstracts Joint Nutrition Symposium*, Antwerp, Belgium, August 21–25, pp. 78.
- Assenza, A., Bergero, D., Tarantola, M., Piccione, G., Caola, G., 2004. Blood serum branched chain amino acids and tryptophan modifications in horses competing in long distance rides of different length. *J. Anim. Physiol. Anim. Nutr.* 88, 172–177.
- Bergero, D., Assenza, A., Attanzio, G., Piccione, G., Velis, A., 2001. Approccio fisiologico-nutrizionale alle modificazioni del peso corporeo, dell'ematocrito e degli elettroliti nel cavallo fondista impegnato in gare di resistenza di lunga durata (RLD). *Atti 3° Convegno "Nuove Acquisizioni in Materia di Alimentazione, Allevamento ed Allenamento del Cavallo Sportivo"*, Campobasso, 12–14 luglio 2001, pp. 103–109.
- Bergero, D., Miraglia, N., Polidori, M., Ziino, M., Gagliardi, D., 2002a. Blood serum and skin fatty acid levels in horses and the use of dietary polyunsaturated fatty acids. *Anim. Res.* 51, 157–163.
- Bergero, D., Miraglia, N., Schiavone, A., Polidori, M., Meineri, G., 2002b. Effect of dietary PUFAs and Vitamin E on serum oxidative status in horses performing very light exercise. *Book of Abstract 53rd Annual Meeting of the European Association for Animal Production*, Cairo, Egypt, 1–4 September, pp. 263. session H.2, Abstract Nr 4.9.
- Bergero, D., Assenza, A., Schiavone, A., Piccione, G., Perona, G., Caola, G., 2003. Amino acid levels in blood serum of horses performing long lasting low intensity exercise. *Proceedings 7th Conference of the European Society of Veterinary and Comparative Nutrition*, Hannover, 3–4 October 2003, pp. 72.
- Bergero, D., Miraglia, N., Schiavone, A., Polidori, M., Meineri, G., 2004. Effect of dietary LC-PUFAs and Vitamin E on serum oxidative status in horses performing very light exercise. *Ital. J. Anim. Sci.* 3, 37–41.
- Burger, D., Dollinger, S., 1998. Raisons d'élimination, état de santé et carrière sportive des chevaux dans les raids d'endurance en Europe et dans les pays arabes: approche statistique. *Prat. Vet. Equine* 30, 91–97.
- Burke, L.M., 2001. Nutritional needs for exercise in the heat. *Comp. Biochem. Physiol., A* 128, 735–748.
- Caola, G., 2001. Fisiologia dell'esercizio fisico del Cavallo. *Ed. Calderini Edagricole*, Bologna, pp. XIV+154.
- Castejon, F., Rubio, D., Tovar, P., Vinuesa, M., Riber, C., 1994. A comparative study of aerobic capacity and fitness in three different horse breeds (Andalusian, Arabian and Anglo-Arabian). *Zentralbl. Veterinärmed.* 41, 645–652.
- Chiaradia, E., Avellini, L., Rueca, F., Spaterna, A., Porcello, F., Antonioni, M.T., Gaiti, A., 1998. Physical exercise, oxidative stress and muscle damage in racehorses. *Comp. Biochem. Physiol., B* 119, 833–836.
- Clayton, H.M., 1991. *Conditioning Sport Horses*. Sport Horse Publications, Saskatoon, Canada, p. 271.
- Demonceau, T., 1992. Chaleur, déshydratation et électrolytes: thermorégulation et exercice physique. *Equathlon* 4, 13–19.
- Dillon, J.C., 1992. Tryptophane, serotonine et appetit spécifique pour les protéines et les glucides. *Cah. Nutr. Diét.* 27, 272–277.
- Duren, S.E., 2000. Feeding the endurance horse. In: Pagan, J.D. (Ed.), *Advances in Equine Nutrition*. Nottingham University Press, Nottingham, pp. 351–363.
- Dusterdieck, K.F., Schott 2nd, H.C., Eberhart, S.W., Woody, K.A., Coenen, M., 1999. Electrolyte and glycerol supplementation improve water intake by horses performing a simulated 60 km endurance ride. *Equine Vet. J.* 30, 418–424 (Suppl.).
- Essen-Gustavsson, B., Gottlieb-Vedi, M., Lindholm, A., 1999. Muscle adenine nucleotide degradation during sub maximal treadmill exercise to fatigue. *Equine Vet. J.* 30, 298–302 (Suppl.).
- Flaminio, M.J., Rush, B.R., 1998. Fluid and electrolyte balance in endurance horses. *Vet. Clin. North Am., Equine Pract.* 14, 147–158.
- Foreman, J.H., 1998. The exhausted horse syndrome. *Vet. Clin. North Am., Equine Pract.* 14, 205–219.
- Frape, D.L., 1988. Dietary requirements and athletic performance of horses. *Equine Vet. J.* 20, 163–172.
- Hargreaves, B.J., Kronfeld, D.S., Waldron, J.E., Lopes, M.A., Gay, L.A., Saker, K.E., Cooper, W.L., Sklan, D.J., Harris, P.A., 2001. Oxidative status of endurance horses. *Proceedings 17th Symposium of the Equine Nutrition and Physiology Society*, Lexington, Kentucky, May 31 June 2, 2001, pp. 280–281.

- Holmann, W., Mader, A., 1999. I limiti della capacità di prestazione dell'organismo umano dal punto di vista fisiologico. *Riv. Cult. Dello Sport SdS* 29, 2–10.
- Hyypä, S., Poso, R.A., 1998. Fluid, electrolyte, and acid–base responses to exercise in racehorses. *Vet. Clin. North Am., Equine Pract.* 14, 121–135.
- Hyypä, S., Saastamoinen, M., Poso, R.A., 1999. Effect of a post exercise fat-supplemented diet on muscle glycogen repletion. *Equine Vet. J.* 30, 493–498 (Suppl.).
- Kronfeld, D.S., 1996. Dietary fat affects heat production and other variables of equine performance, under hot and humid conditions. *Equine Vet. J.* 22, 24–34 (Suppl.).
- Kronfeld, D.S., Crandell, K.M., Custalow, S.E., Ferrante, P.L., Gay, L.A., Graham-Thiers, P., Kline, K.A., Taylor, L.E., Holland, J.L., Wilson, J.A., Sklan, D.J., Harris, P.A., Tiegs, W., 2001. Studies of fat adaptation and exercise. In: Pagan, J.D., Geor, R.J. (Eds.), *Advances in Equine Nutrition*, vol. II. Nottingham University Press, Nottingham, pp. 481–482.
- Mathiason-Kochan, K.J., Potter, G.D., Caggiano, S., Michael, E.M., 2001. Ration digestibility, water balance and physiologic responses in horses fed varying diets and exercised in hot weather. *Proceedings 17th Symposium of the Equine Nutrition and Physiology Society*, Lexington, Kentucky, May 31–June 2, 2001, pp. 261–266.
- McGilvery, R.W., 1975. In: Howald, H., Poortmans, J.R. (Eds.), *Metabolic Adaptation to Prolonged Physical Exercise*. Birkhauser, Basel, pp. 12–30.
- Neumann, G., 1990. La struttura della prestazione negli sport di resistenza. *Riv. Cult. Sportiva SdS* 20, 66–77.
- Newsholm, E.A., Blomström, E., Ekblom, B., 1992. Physical and mental fatigue: metabolic mechanisms and importance of plasma amino acids. *Br. Med. Bull.* 48, 477–495.
- Nyman, S., Jansson, A., Dahlborn, K., Lindholm, A., 1996. Strategies for voluntary rehydration in horses during endurance exercise. *Equine Vet. J.* 22, 99–106 (Suppl.).
- Pagan, J.D., Essen-Gustafsson, B., Lindholm, A., Thornton, J., 1987. The effect of dietary energy source on exercise performance in Standardbred horses. In: Gillespie, J.R., Robinson, N.E. (Eds.), *Equine Exercise Physiology*, vol. 2, pp. 686–700.
- Pinna, W., Assenza, A., Bergero, D., Caola, G., 2004. A multi-factorial approach to the definition of the “performance structure” of the endurance horse: a preliminary report. *Proceedings Second European Equine Health and Nutrition Congress*, Lelystad, Netherlands, 19–20 March 2004, pp. 87–89.
- Prince, A., Geor, R., Harris, P., Hoekstra, K., Gardner, S., Hudson, C., Pagan, J., 2001. Comparison of the metabolic responses of trained Arabian and Thoroughbred horses during high and low intensity exercise. *Proceedings 17th Symposium of the Equine Nutrition and Physiology Society*, Lexington, Kentucky, May 31–June 2, 2001, pp. 267–272.
- Rose, R.J., Arnold, K.S., Church, S., Paris, R., 1980. Plasma and sweat electrolyte concentrations in the horse during long distance exercise. *Equine Vet. J.* 12, 19–22.
- Sacheck, J.M., Blumberg, J.B., 2001. Role of vitamin E and oxidative stress in exercise. *Nutrition* 17, 809–814.
- Schott 2nd, H.C., Hinchcliff, K.W., 1998. Treatments affecting fluid and electrolyte status during exercise. *Vet. Clin. North Am., Equine Pract.* 14, 175–204.
- Schott 2nd, H.C., McGlade, K.S., Molander, H.A., Leroux, A.J., Hines, M.T., 1997. Body weight, fluid, electrolyte, and hormonal changes in horses competing in 50- and 100-mile endurance rides. *Am. J. Vet. Res.* 58, 303–309.
- Schott 2nd, H.C., Dusterdieck, K.F., Eberhart, S.W., Woody, K.A., Refsal, K.R., Coenen, M., 1999. Effects of electrolyte and glycerol supplementation on recovery from endurance exercise. *Equine Vet. J.* 30, 384–393 (Suppl.).
- Snow, D.H., Baxter, P., Rose, R.J., 1981. Muscle fibre composition and glycogen depletion in horses competing in an endurance ride. *Vet. Rec.* 108, 374–378.
- Snow, D.H., Mason, D.K., Ricketts, S.W., Douglas, T.A., 1983. Post-race blood biochemistry in Thoroughbreds. *Equine exercise physiology. Proceedings of the First International Conference*, pp. 389–399.
- Sosa Leon, L.A., 1998. Treatment of exercise-induced dehydration. *Vet. Clin. North Am., Equine Pract.* 14, 159–173.
- Spangfors, P., 2000. Some aspects of feeding the endurance horse. In: Pagan, J.D. (Ed.), *Advances in Equine Nutrition*. Nottingham University Press, Nottingham, pp. 341–349.
- Weineck, J., 1990. *Manual d'Entraînement*. Vigot, Paris, pp. 424.
- White, A., Estrada, M., Walzer, K., Wisnia, P., Filgueira, G., Valdés, F., Araneda, O., Behn, C., Martinez, R., 2001. Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. *Comp. Biochem. Physiol., A* 128, 99–104.